THE UNIVERSITY OF ALBERTA

STUDIES ON CARBON MONOXIDE POISONING IN CHICKS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

BY

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ABSTRACT

Experiments in which chicks were exposed to various atmospheric concentrations of carbon monoxide were performed. Carbon monoxide concentrations in the atmosphere and per cent carboxyhemoglobin in the blood necessary to kill chicks were determined. The symptoms and gross and histological lesions observed as a result of the carbon monoxide exposure are reported. Colorimetric and gas-liquid chromatographic methods for determining carboxyhemoglobin are described in detail.

A carbon monoxide concentration of 2,000 ppm in air for about two hours was necessary to cause mortality in day-old and 4 week-old chicks, and resulted in carboxyhemoglobin concentrations of at least 60%. There was no evident relationship between high and low hemoglobin levels, which were within the normal range for chicks, and susceptibility to carbon monoxide poisoning.

The symptoms of carbon monoxide poisoning observed were those characteristic of anoxemia, that is, irritability, head shaking, drowsiness, incoordination, dyspnea, clonic spasm and coma. An unusual symptom noted was a tendency for the birds to pick at their toes and wing tips.

Aside from the presence of a pink color in

all of the tissues, no gross pathological lesions of significance were observed. Degeneration of the neurons of the brain and particularly the Purkinje cells of the cerebellum with perivascular and perineuronal edema and stasis of the red blood cells were seen on histopathological examination.

The colorimetric method for carboxyhemoglobin proved to be a satisfactory test. It was effective in testing clotted as well as unclotted blood. The gas-liquid chromatographic method of analysis gave carboxyhemoglobin values considerably below those obtained by the colorimetric method.

ACKNOWLEDGEMENTS

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INTRODUCTION

Since 1952, a number of specimens submitted to the Poultry Disease Section, Alberta Veterinary Laboratory, displayed a syndrome which was diagnosed as carbon monoxide poisoning (Table 1). The condition was characterized by bright red color of the beak and shanks, brighter than usual color of the lungs and blood, and mottling of the kidneys and livers with pale areas intermixed with pink areas and petechial hemorrhages. Blood samples from a number of these specimens were analysed chemically for carboxyhemoglobin (hereafter referred to as HbCO); levels varied from 0% to 35% HbCO. Between 1958 and 1959, there was a drop in the number of specimens which were diagnosed as carbon monoxide poisoning. This resulted from a hesitancy on the part of the pathologists to diagnose carbon monoxide poisoning because of the low HbCO levels found on analysis, and because of insufficient information on blood levels of carbon monoxide in poultry that might be considered to be indicative of carbon monoxide poisoning.

This uncertainty prompted the project described in this thesis. The objectives of the experiments were to:

1. determine the HbCO levels in the blood of

TABLE 1

NUMBERS* OF CARBON MONOXIDE POISONINGS
DIAGNOSED DURING 1952 TO 1961 IN CHICKS AND POULTS
SUBMITTED TO THE VETERINARY LABORATORY,
ALBERTA DEPARTMENT OF AGRICULTURE

Year	Numbers*
1952	20
1953	28
1954	45
1955	85
1956	188
1957	155
1958	118
1959	27
1960	12
1961	31

* Represents groups of birds

young chicks necessary to cause death.

2. determine the carbon monoxide concentrations in air required to attain fatal HbCO levels in chicks.

3. ascertain whether or not the same levels of HbCO in blood and carbon monoxide in air are lethal to chicks and guinea pigs.

4. observe symptoms in chicks resulting from carbon monoxide poisoning.

5. compare the gross pathological lesions in chicks that result from carbon monoxide poisoning with those observed in specimens with the "carbon monoxide syndrome".

6. obtain histological criteria in chicks which would aid in diagnosing carbon monoxide poisoning.

7. devise tests for HbCO which could be used in routine diagnostic work.

REVIEW OF THE LITERATURE

Mode of Action of Carbon Monoxide

The physiological interest in carbon monoxide arises from its property of combining with hemoglobin in a manner similar to oxygen. Combination of carbon monoxide with reduced hemoglobin in the absence of oxygen, and dissociation of HbCO follow the same laws that govern the combination of oxygen with reduced hemoglobin and the dissociation of oxyhemoglobin. The S-shaped curve resulting from the plotting of the partial pressure of the gas against the percentage saturation of the hemoglobin is identical in shape for both gases; variations in pH, temperature and salt content have the same influence in each case (11). The difference lies in the range of effective partial pressures; for example, the hemoglobin of human blood becomes half saturated with oxygen at a partial pressure of about 30 mm., however, it is half saturated with carbon monoxide, under the same conditions of temperature, pH, etc., when exposed to a partial pressure of the gas of only 0.125 mm. (25).

When blood is exposed to a mixture of oxygen and carbon monoxide, the hemoglobin is divided between the two gases, the reaction obeying the law of mass action, that is, the proportion of hemoglobin combined

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with either gas depends on the relative partial pressure of the oxygen and carbon monoxide, and upon a constant (K) which expresses the relative affinity of the blood for the two gases. This relationship is expressed by the equation, $\frac{HbCO}{HbO_2} = \frac{K(CO)}{(O_2)}$ (25).

The equilibrium constant (K) varies considerably between different species (25). K values for some species are: ox, 179 (41); rabbit, 83-140 (25); mice, 118-278 (24); dog, 570 (3); and human, 233-272 (23).

Oxygen combines with hemoglobin about 10 times as fast as does carbon monoxide under similar conditions of pH, temperature and concentration of reagents; however, the great affinity of carbon monoxide for hemoglobin is due to the fact that HbCO dissociates very much more slowly than oxyhemoglobin (25).

The rate at which the percentage saturation of hemoglobin with carbon monoxide increases in different animals is also proportional to the respiratory exchange per unit of body weight. In small animals and birds, respiratory exchange is more rapid than in large animals and as a result, equilibrium is reached sooner in the former (25).

Carbon monoxide acts as a poison by preventing the normal carriage of oxygen in the blood, and not as a direct tissue poison (4, 9, 32, 39). This has been

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demonstrated by experiments in which yeast, isolated tissue cultures and insects were subjected to mixtures of oxygen and carbon monoxide with no adverse effects on metabolism (10, 16, 25).

Animal Experiments

The most extensive investigation of the effects of carbon monoxide on a variety of species was conducted by Burrell and co-workers (7). They subjected several species to concentration of carbon monoxide in air varying from 1,000 to 5,700 parts per million (hereafter referred to as ppm.). The results of these investigations are summarized in Table 2. Only the actions of the subjects under experiment were observed; blood analyses were not performed. The main symptoms observed were respiratory distress, jerking movement of the head and limbs, a gradual loss of control of the appendages, with the subject finally falling and rolling over on its back until dead. The investigations illustrated the variation in susceptibility of individuals of the same species to identical levels of atmospheric carbon monoxide. Some subjects required twenty times as long to show the same amount of distress from exposure to the gas as other individuals of the same species under similar conditions of exposure. The subjects studied were ranked for susceptibility to carbon monoxide as follows: 1. canary,

TABLE 2

EFFECTS OF CARBON MONOXIDE ON VARIOUS SPECIES*

Species	No.	Carbon Monoxide in Air (ppm.)	Exposure Time	Effects
Chicken	2	1000	2 hrs. 45 min.	No distress
"	1	1500	1 hr. 50 min.	No distress
"	4	2000	15-55 min.	All collapsed
"	6	2500	10-40 min.	All collapsed
"	1	5700	5 min.	Collapsed
Pigeon	1	1000	1 hr. 40 min.	Some distress
"	2	2000	1 hr. 15 min.	Some distress
"	3	2500	12 min. 15 min. 2 hrs. 20 min.	One collapsed One more collapsed One survivor
"	1	3000	40 min.	All collapsed
"	2	5000	5-9 min.	All collapsed
Canary	8	1000	3 hrs. 50 min.	Some distress - one died
"	12	2000	4-40 min.	All collapsed
"	5	2500-4000	8-40 min.	One collapsed - no distress in remainder
Sparrow	1	2000	55 min.	No distress
"	1	3000	30 min.	Collapsed
Dog	1	2000	2 hrs.	No distress
"	2	2500	20-25 min.	All collapsed
Guinea pig	1	2000	5 min.	No distress
"	6	2400-2700	29 min.-4 hrs. & 10 min.	Three collapsed Three survived
"	4	3500-3700	12-15 min.	All collapsed
"	8	4000-4600	3-26 min.	All collapsed
"	8	5000	6-21 min. 6 hrs. 30 min.	Seven collapsed One survived
Mouse	7	1000	1 hr. 15 min.-4 hrs. 20 min.	Only one showed distress
"	6	2000	15-40 min.	Some distress-none collapsed
"	2	3500	6 min.	All collapsed

* Burrell _et al._ (7)

2. mouse, 3. chicken, 4. small dog, 5. pigeon, 6. English sparrow, 7. guinea pig, 8. rabbit.

A summary of other studies on carbon monoxide poisoning in various kinds of animals and birds is presented in Table 3.

Pathological Effects of Carbon Monoxide Anoxemia

The most consistent lesions reported in carbon monoxide poisoning in animals and humans have been those involving the central nervous system. The lower centers of the telencephalonic division, particularly the caudate and lentiform nuclei of the corpus striatum appear to be most frequently affected. In the cerebellum, the Purkinje cells are often affected. The nerve cells of the cortex of the telencephalon do not appear to be as susceptible as the lower coordination centers of this portion of the brain (4, 9, 12, 13, 15, 18, 19, 25, 28, 29, 45).

Yant <u>et al.</u> (52) found dilated blood vessels, petechial hemorrhages and severely damaged nerve cells in dogs subjected to high concentrations of carbon monoxide for short periods of time. Exposure to lower concentrations of the gas for longer periods of time produced pathological changes which were more severe than those observed in the animals exposed to high concentrations for short periods.

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TABLE 3

SUMMARY OF OTHER STUDIES ON EFFECTS OF CARBON MONOXIDE ON VARIOUS KINDS OF ANIMALS AND BIRDS

Carbon Monoxide in Air (ppm.)	Exposure Time	Effects	Per Cent HbCO	Reference Number
		Chicken Experiments		
High	45-90 sec.	Death	46-65	(6)
4000	79-162 min.	Death	--	(42)
High	Short	Death	--	(5)
		Dog Experiments		
6000	6 min.	Death	75-85	(52)
1800-2200	8-16 hrs.	Death	--	(52)
2000-3000	--	Moribund	--	(26)
100	$5\frac{1}{2}$ hrs. daily - 11 weeks	Apparently healthy	20	(28)
800-1000	6-8 hrs. daily - 36 weeks	Apparently healthy	--	(48)
4000	30 min.	Increased respiration and pulse	50	(30)
3000	76 min.	Death	61-88	(40)
10000	16-25 min.	Death	80-90	(44)
		Guinea Pig Experiments		
8000	50 min.	Death	84	(3)
1500	Less than 3 days	Death	--	(8)
		Rat Experiments		
1500	Less than 3 days	Death	--	(8)
500	4-6 hrs.	Depressed metabolic rate	--	(47)
		Mice Experiments		
1000-1200	10 min.	Respiratory distress	--	(51)
1500	Few hours	Death	61-75	(24)
1500	Less than 3 days	Death	--	(8)

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The telencephalon of the bird is composed entirely of areas which constitute the corpus striatum of mammals. The avian species do not have a cerebral cortex; the numerous strial areas constitute the highest coordinating centers in the brain. In addition the cerebellum of birds compared to mammals is remarkably well-developed (22, 35, 37).

The fact that the highest centers of nervous coordination in avian species are the same areas in which the dominant pathological lesions are observed in carbon monoxide poisoning in mammals, suggested that a histological examination of these areas of the brain would be advisable.

Gas Chromatography for Blood Gas Analysis

Recently Ramsey (38) has called attention to the applicability of gas-liquid chromatography (hereafter referred to as gas chromatography) to the analysis of gases contained in biological fluids. Using this technique, he demonstrated that the oxygen extracted from water and plasma could be measured with ease and great accuracy. Several workers (20, 31, 49, 50) have adapted the methods of gas chromatography to the determination of blood gases as extracted from whole blood. Wilson (49) suggests that gas chromatography is the first reliable method for blood gas analysis that has

been introduced since the classical contributions of Van Slyke in the 1920's. Harris (17) has written an excellent review on the principles of gas chromatography.

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MATERIALS AND METHODS

Sources of Experimental Stock

The chicks used in the experiments were New Hampshire-White Plymouth Rock crosses obtained from the University Farm as day-old chicks. Some White Leghorn chicks from a laying flock maintained at the Veterinary Laboratory were also used. The guinea pigs were from stock kept at the Veterinary Laboratory.

Gas Administration Equipment

This apparatus (Figure 1) consisted of a stainless steel chamber of 6-cubic foot capacity with a glass front and a foam rubber sealed opening at the top, which permitted access to the chamber. The gas was administered through a ½-inch perforated copper pipe located across the back, near the top of the chamber. An exhaust pipe from the chamber was vented to the outside of the building. Air was pumped to the chamber through a flowmeter calibrated to deliver 34,426 ml. of air per minute at maximum flow. The carbon monoxide gas* used had a minimum purity guarantee of 99.5%. The carbon monoxide gas was delivered to the air stream just prior to entering the chamber from a gas cylinder through a flowmeter, capable of delivering

*Matheson Co., Inc., East Rutherford, New Jersey.

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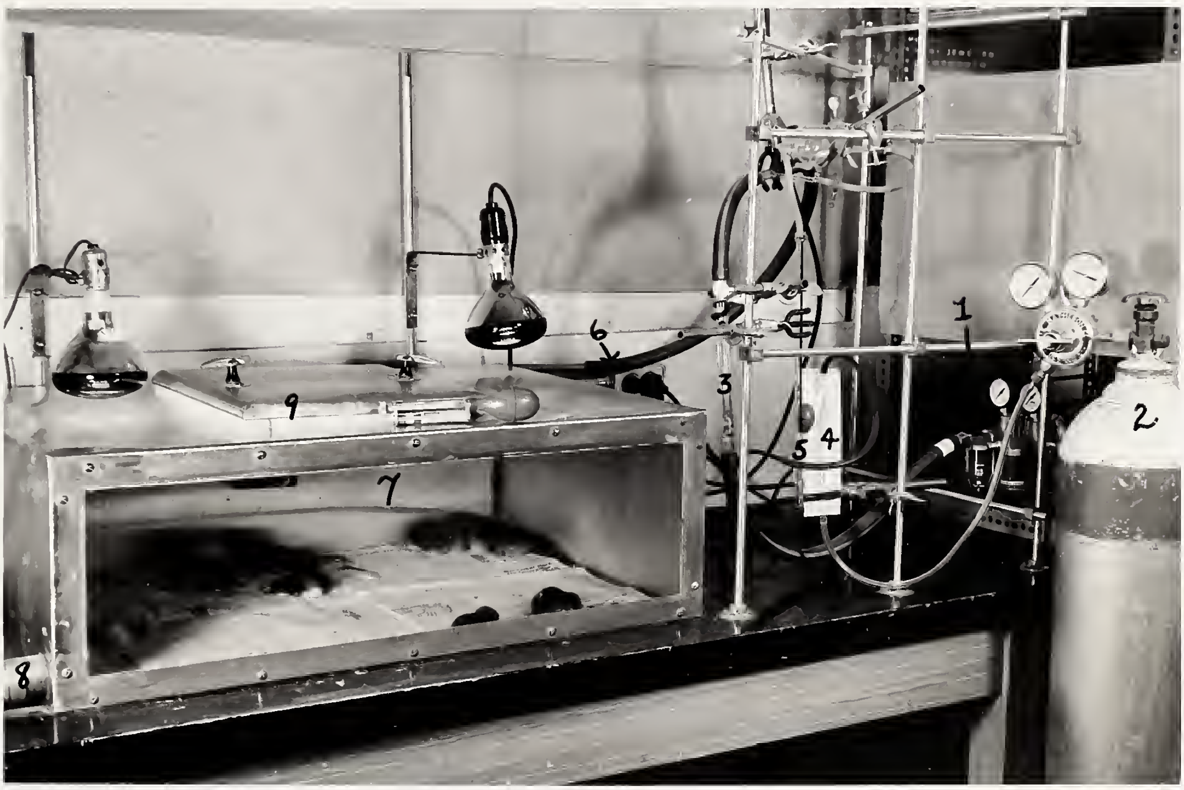
FIGURE 1

EQUIPMENT USED FOR
EXPOSING CHICKS AND GUINEA PIGS TO VARIOUS
CONCENTRATIONS OF CARBON MONOXIDE GAS
IN AIR



1. Air supply. 2. Carbon monoxide supply. 3. Air flowmeter. 4. Carbon monoxide flowmeter. 5. Soap bubble manometer. 6. Air and carbon monoxide stream. 7. Exposure chamber. 8. Exhaust pipe. 9. Foam rubber sealed lid.

5.9 to 426 ml. of the gas per minute. The calibration of the carbon monoxide entering the chamber was accomplished by a soap bubble manometer. The rate of flow of carbon monoxide was determined by timing the flow of soap bubbles up the manometer with a stop watch. The concentration of carbon monoxide gas in air was calculated from the flow through the soap bubble manometer and the constant volume of air delivered by the pump. The temperature in the chamber was maintained, at the optimum for the age of the chicks confined, by two heat lamps suspended over the chamber and turned on and off as required.

Collection of Blood

Blood collection in the chicks was by heart puncture through the anterior aperture of the thoracic cavity. Collection of blood from guinea pigs was by heart puncture between the ribs on either side of the thoracic cavity. Sodium citrate solution (1 gram sodium citrate/100 ml. of distilled water) was used as an anticoagulant at the rate of 2 to 3 drops for each 2 ml. of blood collected. In the case of subjects that died in the chamber, the thoracic cavities were opened and blood was collected from the heart and major vessels. Clotted blood was ground in a test tube-type tissue grinder.

Hemoglobin Determinations

The photoelectric acid hematin test (27) using a Klett-Summerson* instrument was employed for the determination of hemoglobin.

Carboxyhemoglobin Determinations

Two methods for the determination of HbCO in blood were employed.

A. Colorimetric Method

A modification of the method of Waggoner *et al.* (46) was used. In this method, carbon monoxide gas is liberated from blood in a gas sampling flask containing air by a ferricyanide reagent. The air containing carbon monoxide is drawn from the flask through an indicator tube+ by a rubber-bulb aspirator. In the mid-region of the tube, an area impregnated with pallidous silicomolybdate changes color from yellow through green to blue in direct proportion to the concentration of carbon monoxide present. A color standard is supplied with the indicator tubes by the manufacturer. Blood saturated with carbon monoxide (representing 100% HbCO) and 1:1 serial dilutions of same (representing

*Klett Manufacturing Company, New York, U.S.A.

+Mine Safety Appliance Company, Toronto, Ontario.

50, 25 and 12.5% HbCO) were compared against this color standard to give a calibrated color chart which could be used in estimating the amount of HbCO in blood samples of unknown carbon monoxide content. Details of the method are described in the Appendix.

B. Gas Chromatographic Method

A Burrell Kromo-Tog II instrument was used in the gas chromatographic analyses of the blood for carbon monoxide. A special reaction chamber was designed and connected in the carrier flow stream ahead of the column. Oxygen, nitrogen and carbon monoxide gas were released from blood by a ferricyanide reagent in a helium atmosphere in the reaction chamber, and separated in a column packed with molecular sieve. Blood samples saturated with carbon monoxide were analysed and the carbon monoxide peak areas were obtained by planimeter measurements. The average peak area, so obtained, was taken to represent 100% HbCO saturation. This value was used as a standard for comparing peak areas obtained from blood samples of unknown carbon monoxide content. Details of the method are described in the Appendix.

<u>Control Birds and Animals</u>

Control groups of chicks and guinea pigs were kept in rooms which were tested periodically for carbon

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monoxide gas with an air sampling device*. In all cases, these tests were negative. Groups of control birds and animals were sacrificed at intervals and blood examinations conducted for HbCO by the colorimetric method with negative results. Brains were collected from birds to obtain normal tissues for comparative histological examination.

Histological Methods

Brains were removed from at least two birds from each experiment immediately upon death and preserved in 10% formalin solution. These were prepared in standard solutions of an Autotechnicon+, embedded in paraffin and sectioned by generally accepted techniques (1). The sections were mounted and stained with hematoxylin-eosin stain and examined for histopathological lesions.

*Mine Safety Appliance Company, Toronto, Ontario.

+Technicon Company, Chauncey, New York.

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RESULTS AND DISCUSSION

Hemoglobin Values

The hemoglobin values obtained (Table 4) agree with those found by other workers using the photoelectric acid hematin method (2, 34).

Exposure Experiments

A summary of the ten carbon monoxide exposure experiments conducted is shown in Table 5. The data indicate that chicks can tolerate low concentrations of carbon monoxide in air (160 ppm.) for fairly long period of time (7 days) with no outward evidence of distress. Carboxyhemoglobin levels in such chicks were of the order of 7 to 12%. At a somewhat higher exposure level (600 ppm.) symptoms of carbon monoxide poisoning became apparent after about 30 minutes of exposure and HbCO values of 25 to 50% were noted. Death from carbon monoxide, however, did not occur until exposure levels of 2,000 ppm. of carbon monoxide in air were used. At this concentration first deaths occurred after about 2 hours of exposure; HbCO levels noted in chicks that died or were killed after approximately 2 to 4 hours of exposure to this concentration of carbon monoxide ranged from 63 to 75%. Exposure of chicks to 3,600 ppm. of carbon monoxide in air caused

TABLE 4

HEMOGLOBIN VALUES*

Experiment	Age	No.	Mean Hemoglobin	Range
2	6 weeks	8	9.60	8.07 - 10.70
3	6 weeks	8	9.43	8.70 - 10.06
4	4 weeks	10	8.92	8.00 - 9.37
5	4 weeks	10	9.58	8.90 - 10.35
6	Day old	10	9.63	8.80 - 10.79
7	4 weeks	10	9.82	8.96 - 10.27
8	4 weeks	10	9.48	7.00 - 11.57

* Values expressed in grams of hemoglobin per 100 ml. of blood

TABLE 5

SUMMARY OF EXPERIMENTS ON THE EFFECTS OF EXPOSING CHICKS AND GUINEA PIGS TO VARIOUS CONCENTRATIONS OF CARBON MONOXIDE FOR VARYING PERIODS OF TIME

Chicks	
Experiment 1 - 160 to 970 ppm. carbon monoxide	
25 7-day old chicks HbCO* Colorimetric - A - 7-12 B - 7-12 C - 12-31 D - 25-65 E - 25-65	A - 5 birds removed after 4 days at 160 ppm. - no effects B - 5 birds removed after 7 days at 160 ppm. - blood clotted overnight and tested C - 5 birds removed after 9 days at 160 ppm. and 2 days at 650 ppm. - skin pink, otherwise normal D - 5 birds removed after 9 days at 160 ppm., 6 days at 650 ppm. and 2 days at 970 ppm. - eating, drowsy, irritable E - 5 birds removed after 9 days at 160 ppm., 6 days at 650 ppm. and 7 days at 970 ppm. - eating, drowsy, eyes watering
Experiment 2 - 200 ppm. carbon monoxide	
8 6-week old chicks HbCO Colorimetric - 7-12	80 min. - birds eating and drinking normally
Experiment 3 - 600 ppm. carbon monoxide	
8 6-week old chicks HbCO Colorimetric - 25-50	27 min. - picking toes, diarrhoea 34 min. - gasping 60 min. - active. Bled from heart and allowed to remain in room atmosphere for one hour - all normal
Experiment 4 - 1000 ppm. carbon monoxide	
10 4-week old chicks HbCO Colorimetric - 50-63 Gas chromatographic - 22.6-40.4	90 min. - picking toes, gasping, diarrhoea 152 min. - drowsy, huddling 212 min. - harvested blood - all birds alive

* Per cent saturation carboxyhemoglobin

Experiment 5 - 1650 ppm. carbon monoxide	
10 4-week old chicks HbCO Colorimetric - 31-50	10 min. - shaking heads 20 min. - picking toes, gasping, diarrhoea 95 min. - one bird dead - others normal when removed
Experiment 6 - 2000 ppm. carbon monoxide	
10 day-old chicks HbCO Colorimetric - 63-75	11 min. - gasping, incoordination 21 min. - toe picking started 71 min. - jerking movements 110 min. - first bird died 311 min. - last bird moribund, sacrificed
Experiment 7 - 2000 ppm. carbon monoxide	
10 4-week old chicks HbCO Colorimetric - 63-75 Gas chromatographic - 46.7-53.6	14 min. - gasping 31 min. - toe picking started 120 min. - one dead 280 min. - remainder clonic spasms, sacrificed moribund
Experiment 8 - 3600 ppm. carbon monoxide	
10 4-week old chicks HbCO Colorimetric - 63-75	8 min. - picking toes 14 min. - all gasping and staggering 35 min. - eight dead 45 min. - all dead - blood collected

Guinea pigs	
Experiment 9 - 2000 to 4000 ppm. carbon monoxide	
5 3-month old guinea pigs HbCO Colorimetric - 63-75	40 min. - 2000 ppm. - drowsy, one prone 195 min. - raised gas flow to 3000 ppm. 225 min. - gasping, clonic spasm head and legs - feet and ears pink 290 min. - gas flow 4000 ppm. - severe dyspnea 360 min. - all moribund and bled
Experiment 10 - 4200 ppm. carbon monoxide	
5 3-month old guinea pigs HbCO - Colorimetric - 63-81 Gas chromatographic - 47.3-55.3	15 min. - all gasping, jerking, thrashing 68 min. - all dead when removed, blood collected

a high percentage of the chicks to die within the first half hour of exposure and resulted in HbCO concentrations similar to those obtained by exposing chicks to 2,000 ppm. for 2 to 4 hours. It would, therefore, appear from these results that while exposing chicks to moderate concentrations of carbon monoxide (600 ppm.) does produce symptoms of distress, fairly high levels (about 2,000 ppm.) are required to produce mortality attributable to carbon monoxide poisoning and HbCO levels in such chicks are in excess of 60%.

The data also indicate that chicks exposed to moderate levels of carbon monoxide (600 ppm.) in air, and possessing moderately high HbCO levels (25 to 50%), recover rapidly when removed from their carbon monoxide environment to a normal air environment.

During the course of the study, wide variations in susceptibility of different individuals to carbon monoxide poisoning were noted. The differences in susceptibility to carbon monoxide poisoning did not appear to be related to hemoglobin level; chicks with high hemoglobin levels were not any more resistant to carbon monoxide poisoning than those with low hemoglobin levels. The latter observation agrees with the findings of Schwerma *et al.* (40) that no direct relationship exists between the concentration of hemoglobin in the

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blood of dogs and the chance of survival from carbon monoxide asphyxia.

Carboxyhemoglobin levels noted in guinea pigs, that died as a result of exposure to carbon monoxide in air, were similar to those noted in chicks that died from exposure to carbon monoxide; lethal levels ranged from 63 to 81%.

Comparison of Colorimetric and Gas Chromatographic HbCO Methods

A comparison of HbCO results obtained colorimetrically and gas chromatographically (Table 5) indicates that the gas chromatographic method of HbCO analysis yields values considerably below those obtained by the colorimetric test. No satisfactory explanation for this can be offered.

The colorimetric test proved to be rapid and repeatable. At high levels of HbCO saturation, the test was difficult to interpret since only slight differences in shade of color occurred. On the other hand, at low levels of HbCO, marked differences in color occurred which made comparisons with the calibrated color standards relatively easy.

The colorimetric test proved satisfactory for testing clotted blood as well as unclotted blood. Most of the specimens received in diagnostic laboratories have

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been dead for a period of one or more days, and the blood is clotted. Under these circumstances, a test which permits testing of clotted blood is advantageous, particularly since it has been shown (14) that HbCO is unaffected by clotting, putrefaction and storage of the blood for periods as long as 35 years.

Symptoms

The symptoms observed were those expected to result from anoxemia. The only unusual symptom noted was a tendency of the chicks to pick at their toes and wing tips. The sequence of symptoms observed in the birds subjected to various concentrations of carbon monoxide are summarized in Table 6.

Pathological Lesions

The only gross pathological lesion noted was a pink color in all tissues. No mottling or petechial hemorrhages characteristic of "carbon monoxide syndrome" were observed in chicks exposed to carbon monoxide, whether examined freshly killed or after storage overnight under refrigeration.

Circulatory changes involving dilation of the vessels with a stasis of red blood cells and perivascular and perineuronal edema were observed (Figure 2). Degeneration of the neuronal cells of the strial area and the Purkinje cells was evidenced by swelling,

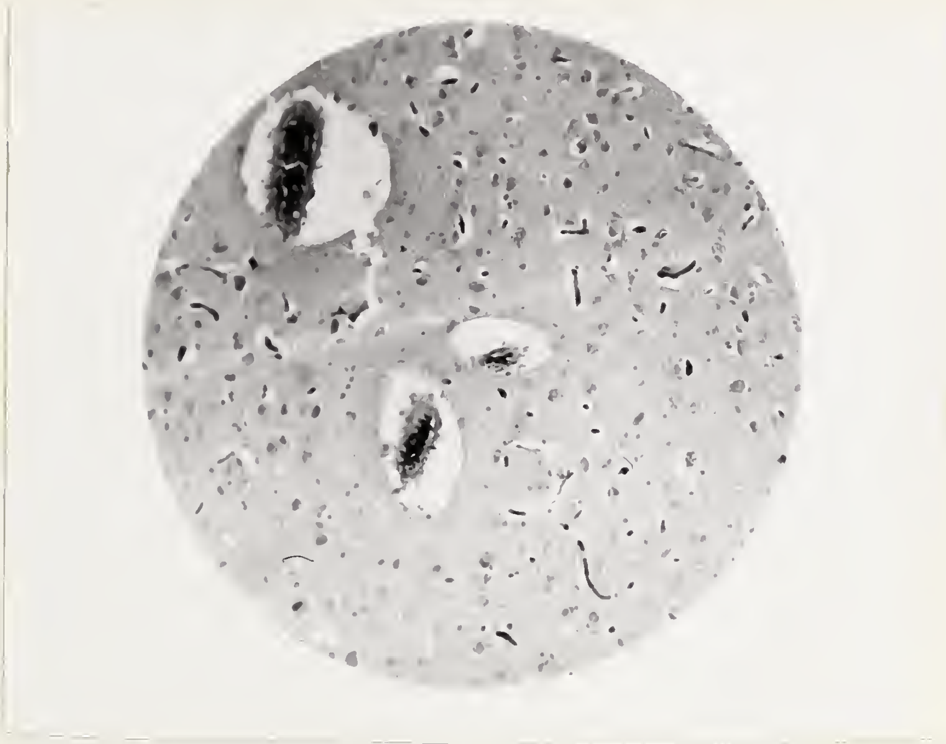
TABLE 6

SUMMARY OF SYMPTOMS OBSERVED IN CHICKS SUBJECTED TO AIR CONTAINING VARIOUS CONCENTRATIONS OF CARBON MONOXIDE

	Skin pink	Up to 1000 ppm.
	Irritability	Up to 1000 ppm.
	Eupnia	Up to 1000 ppm.
	Diarrhoea	Up to 1000 ppm.
1000 ppm.	Head shaking	2000 ppm.
1000 ppm.	Polypnea	2000 ppm.
1000 ppm.	Drowsiness	2000 ppm.
1000 ppm.	Toe picking	2000 ppm.
1000 ppm.	Incoordination	2000 ppm.
3000 ppm. and up	Extreme dyspnea	2000 ppm.
3000 ppm. and up	Clonic spasm	2000 ppm.
3000 ppm. and up	Coma	
3000 ppm. and up	Death	

FIGURE 2

PHOTOMICROGRAPH SHOWING STRIAL AREA
OF A FOUR-WEEK OLD CHICK



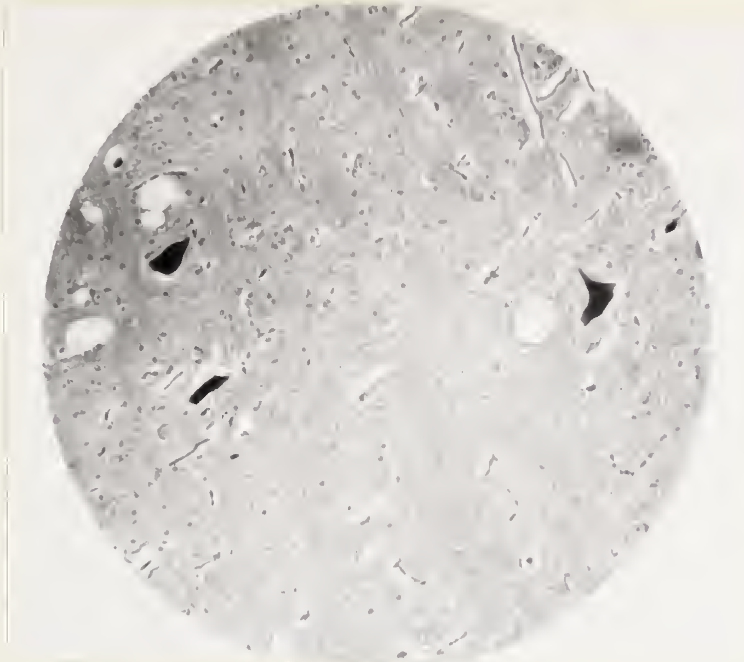
From chick exposed to carbon monoxide, Experiment 1 E, (x100). Note perivascular edema.

chromatolysis and vacuolation (Figures 3 & 4). In less severely affected cells, clumping of chromatin material gave a granular appearance. There appeared to be severe effects in some areas of the brain with contiguous areas being apparently unaffected. The normal location of the Purkinje cells between the outer molecular layer and the inner granular layer of the parenchyma of the cerebellum was occupied by an incomplete layer of cells which had degenerated (Figure 5).

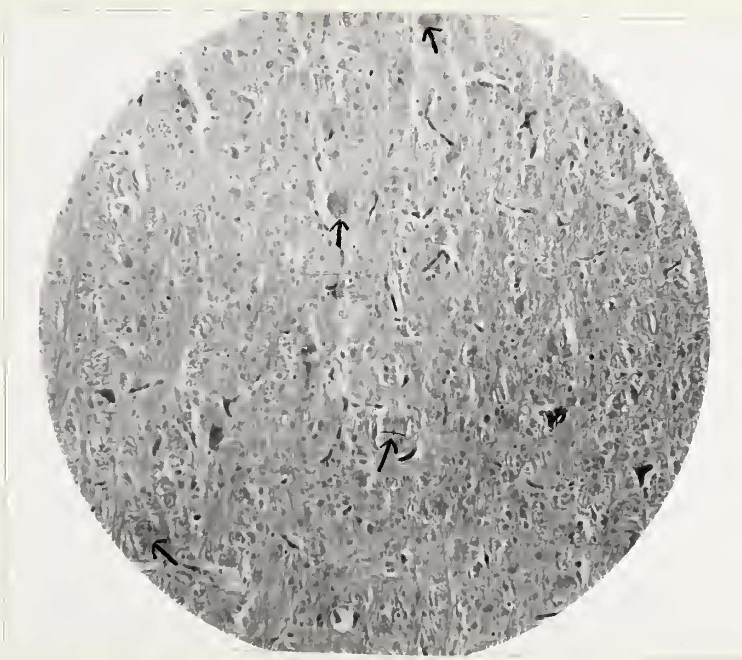
Histopathological lesions similar to those observed in brain tissue of carbon monoxide affected chicks may be found in a number of other diseases afflicting the central nervous system. Degeneration of the neurons, blood stasis, perineuronal and perivascular edema have been reported in Newcastle disease, avian encephalomyelitis, anoxemia from other causes and avian encephalomalacia, resulting from Vitamin E deficiency (9, 21, 33, 36, 43).

FIGURE 3

PHOTOMICROGRAPHS SHOWING STRIAL AREAS
OF FOUR-WEEK OLD CHICKS



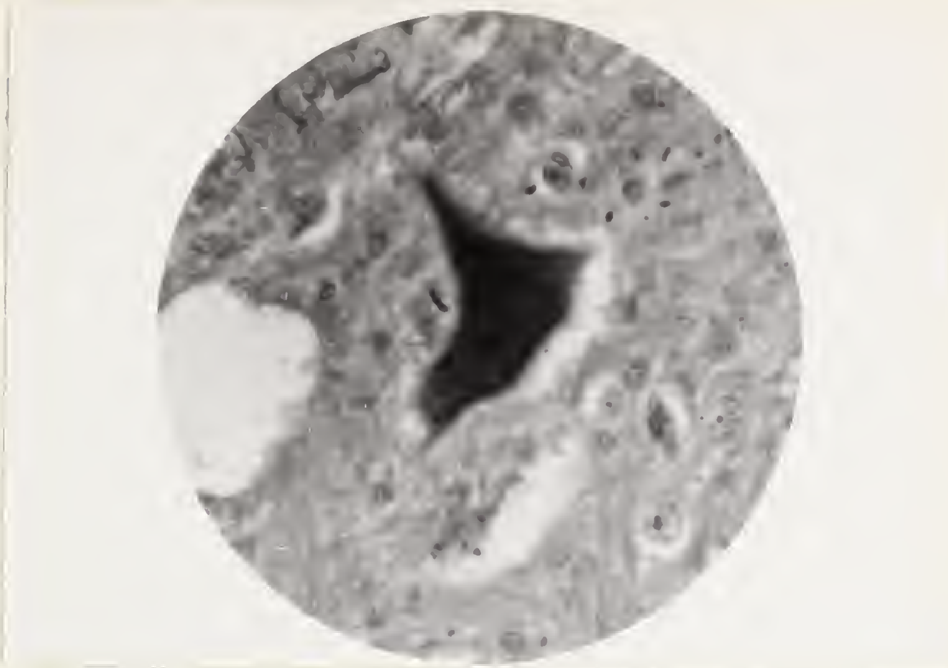
A. Normal (x100).



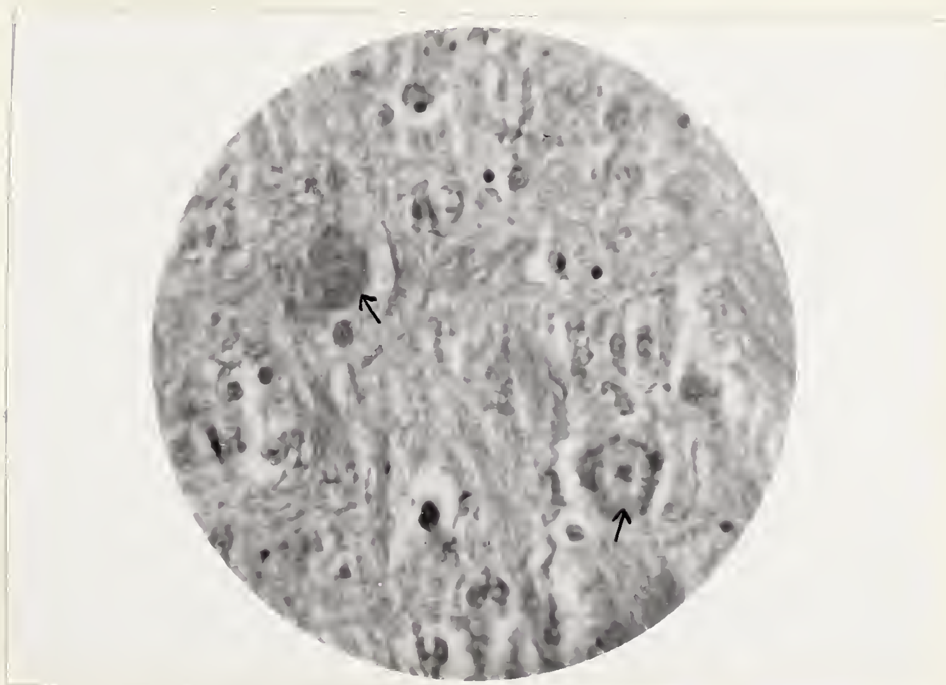
B. From chick exposed to carbon monoxide, Experiment 1 E (x100). Note degeneration of neurons.

FIGURE 4

PHOTOMICROGRAPHS SHOWING STRIAL AREAS
OF FOUR-WEEK OLD CHICKS



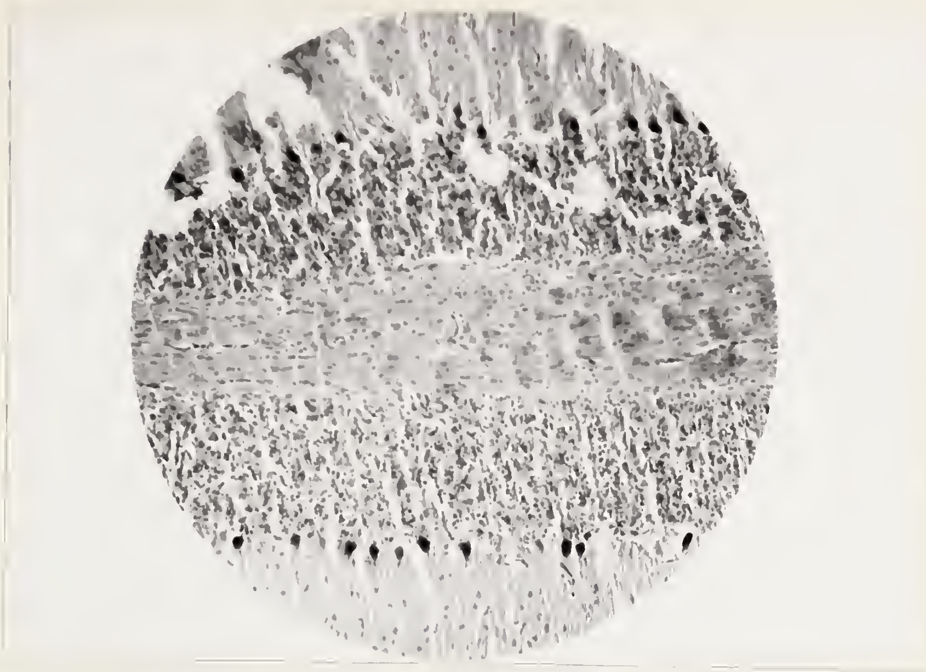
A. Normal (x500).



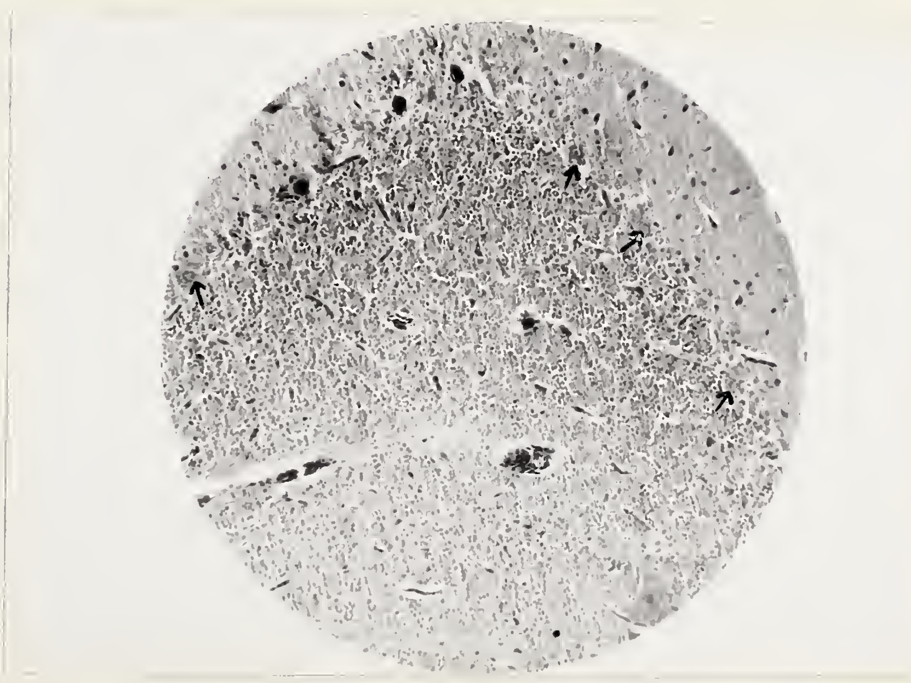
B. From chick exposed to carbon monoxide, Experiment 1 E (x500). Note degeneration of neurons.

FIGURE 5

PHOTOMICROGRAPHS SHOWING CEREBELLA
OF FOUR-WEEK OLD CHICKS



A. Normal (x100). Striated appearance an artefact of sectioning.



B. From chick exposed to carbon monoxide, Experiment 1 E (x100). Note degeneration of Purkinje cells.

SUMMARY AND CONCLUSIONS

It has already been indicated (see Review of the Literature) that high concentrations of carbon monoxide in the atmosphere and correspondingly high levels of carboxyhemoglobin in the blood of many species are required to cause death. In these experiments, chicks did not deviate greatly from mammalian species in their capacity to withstand carbon monoxide gas. Death from carbon monoxide occurred when exposure levels of 2,000 ppm of carbon monoxide in air were used. At this concentration, first deaths occurred after about two hours of exposure. Carboxyhemoglobin levels noted in chicks that died or were killed after approximately 2 to 4 hours of exposure to this concentration of carbon monoxide ranged from 63 to 75%. In the light of these findings, it would seem that the carbon monoxide diagnoses made in the past on the basis of the so-called "carbon monoxide syndrome", along with 5 to 10% carboxyhemoglobin levels, were in error.

Wide variations in susceptibility of different individuals to carbon monoxide poisoning were noted. Differences in susceptibility to carbon monoxide poisoning did not appear to be related to differences in hemoglobin levels.

The symptoms of carbon monoxide poisoning observed were those expected to result from anoxemia,

that is, irritability, head shaking, drowsiness, incoordination, dyspnea, clonic spasm and coma. An unusual symptom observed was a tendency for the chicks to pick at their toes and wing tips.

The only gross pathological lesion observed in the experimental subjects was pink color in the tissues. No mottling or petechial hemorrhages characteristic of the "carbon monoxide syndrome" was evident. The histopathological lesions observed in brain were associated with circulatory changes involving dilation of the vessels with stasis of red blood cells and perivascular and perineuronal edema. There was degeneration of the neuronal cells of the strial area and the Purkinje cells of the cerebellum. These lesions could not be considered as pathogonomic for carbon monoxide poisoning, inasmuch as they are observed in a number of other diseases afflicting the central nervous system.

The colorimetric method of carboxyhemoglobin determination proved to be rapid, repeatable and satisfactory for testing clotted blood as well as unclotted blood. The gas-liquid chromatographic method of carboxyhemoglobin analysis consistently gave values below those obtained by the colorimetric method.

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APPENDIX

Carboxyhemoglobin Determinations

Details of the colorimetric and gas chromatographic methods used for determining carbon monoxide concentrations in the blood follow.

A. Colorimetric Method

1. The reagent consisted of a mixture of 0.3 grams of saponin, 0.8 grams of potassium ferricyanide, 0.4 ml. of lactic acid, and 0.3 ml. caprylic alcohol (hereafter called ferricyanide reagent). These materials were placed in a beaker and sufficient distilled water was added to bring to 100 ml. The solution was made fresh daily.

2. As much air as possible was evacuated with a water aspirator from a 250 ml. gas sampling flask equipped with stopcocks.

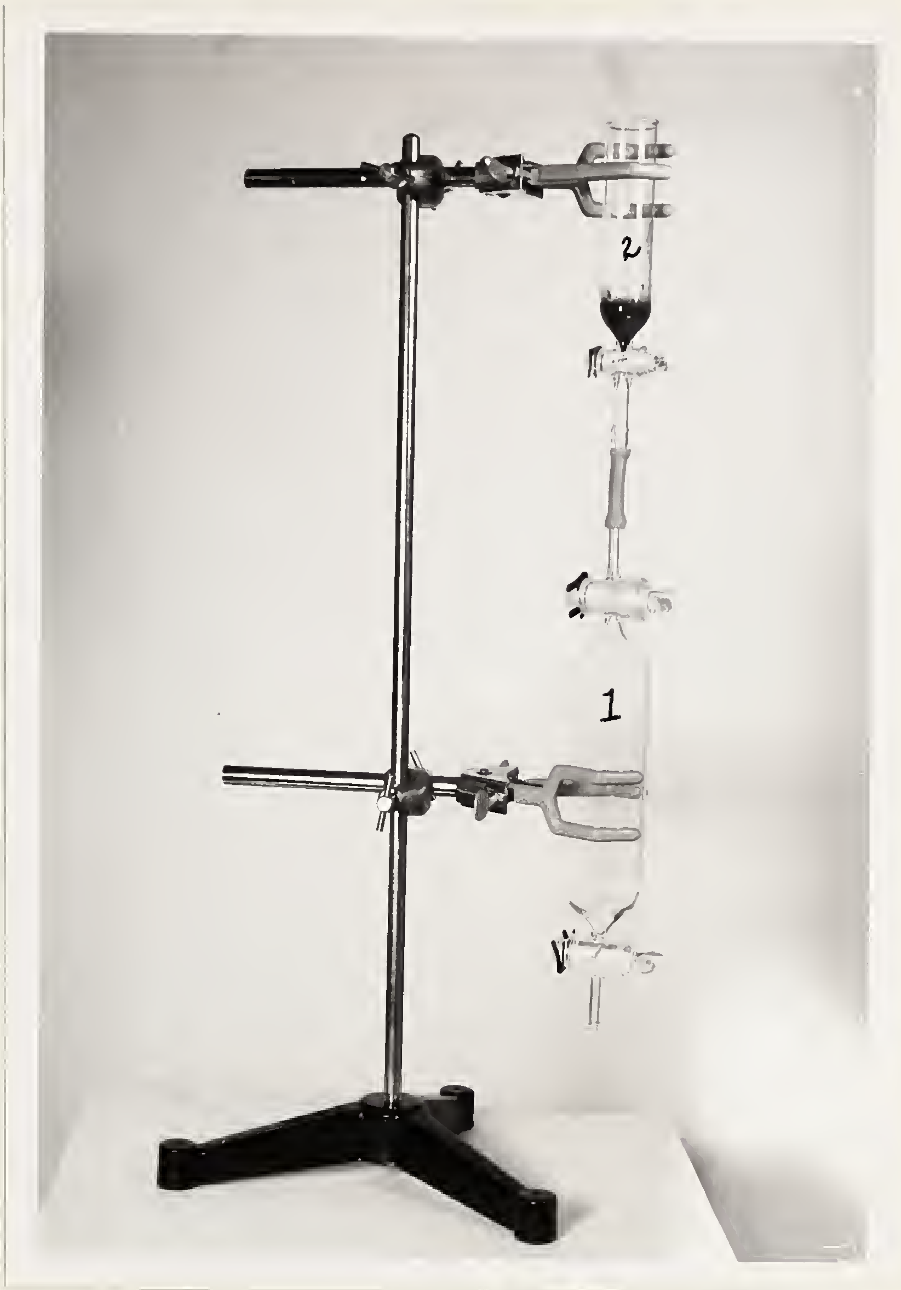
3. One ml. of whole blood was placed in a glass funnel arranged over the flask (Figure 6).

4. Five ml. of the ferricyanide reagent was pipetted into the funnel.

5. With the bottom stopcock of the flask closed, the stopcocks at the top of the flask and the bottom of the funnel were opened. This allowed the blood and ferricyanide reagent to be drawn into the flask.

FIGURE 6

ILLUSTRATION OF PART OF THE APPARATUS USED IN CARBOXYHEMOGLOBIN DETERMINATION BY THE COLORIMETRIC METHOD



1. Gas sampling flask

2. Funnel

6. The stopcock at the top of the flask was closed and the flask was removed and shaken for five minutes causing carbon monoxide in the blood to be liberated as gas.

7. A carbon monoxide indicating tube in a sampling device*, designed for the detection of carbon monoxide in air, was attached to one end of the flask (Figure 7).

8. Both stopcocks on the collecting flask were opened. A 60-ml. rubber-bulb aspirator was attached to the indicating tube, collapsed, and released, allowing the bulb to slowly fill by drawing air from the flask through the carbon monoxide indicating tube. This aspiration procedure was repeated from one to four times depending on the concentration of carbon monoxide present in the gas sampling flask. The carbon monoxide containing air drawn through the indicating tube changed the color of a test area impregnated with pallidous silicomolybdate from yellow through green to blue, directly in proportion to the concentration of the carbon monoxide gas present (Figure 8).

In order to permit comparisons between bloods of unknown HbCO content it was necessary to determine the color changes produced by known concentrations of

*Mine Safety Appliance Company, Toronto, Ontario.

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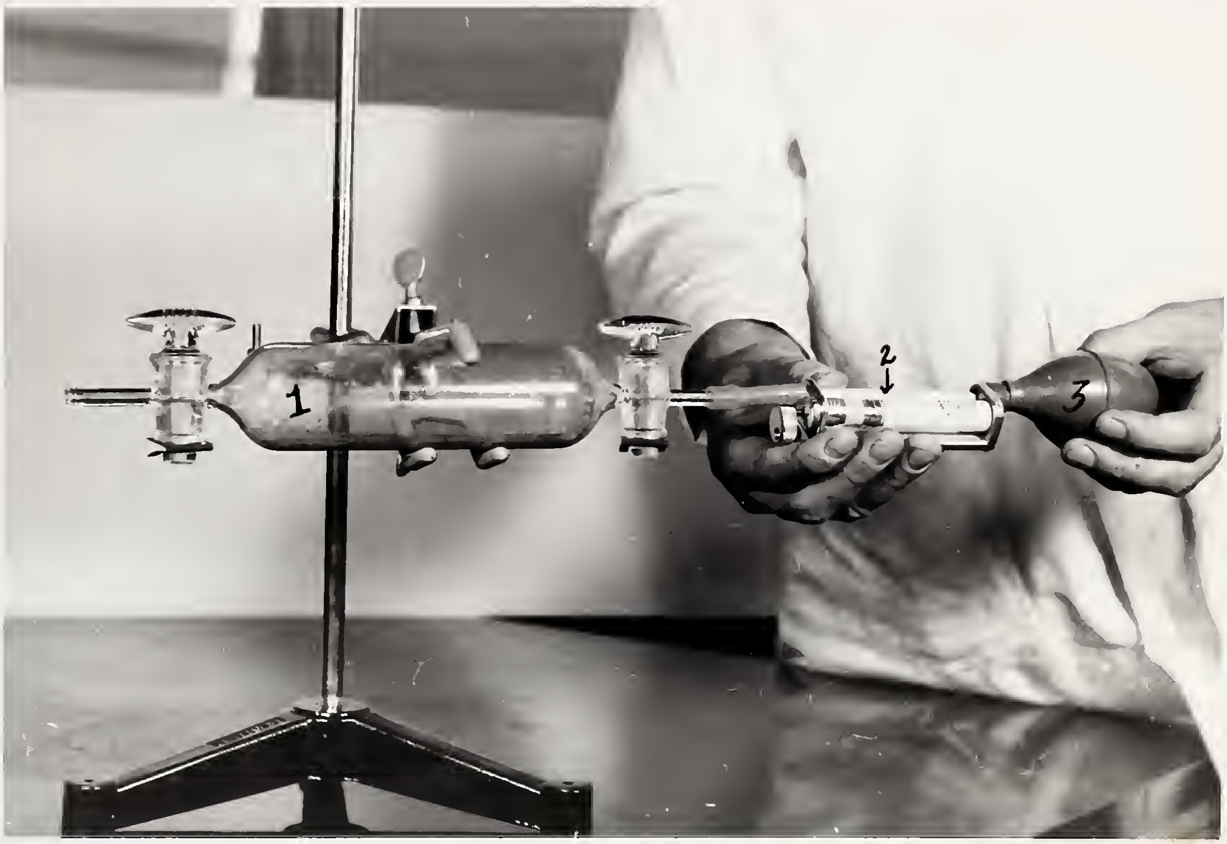
FIGURE 7

ILLUSTRATES THE TECHNIQUE OF TRANSFERRING THE ATMOSPHERE IN THE GAS SAMPLING FLASK THROUGH THE COLORIMETRIC INDICATING TUBE



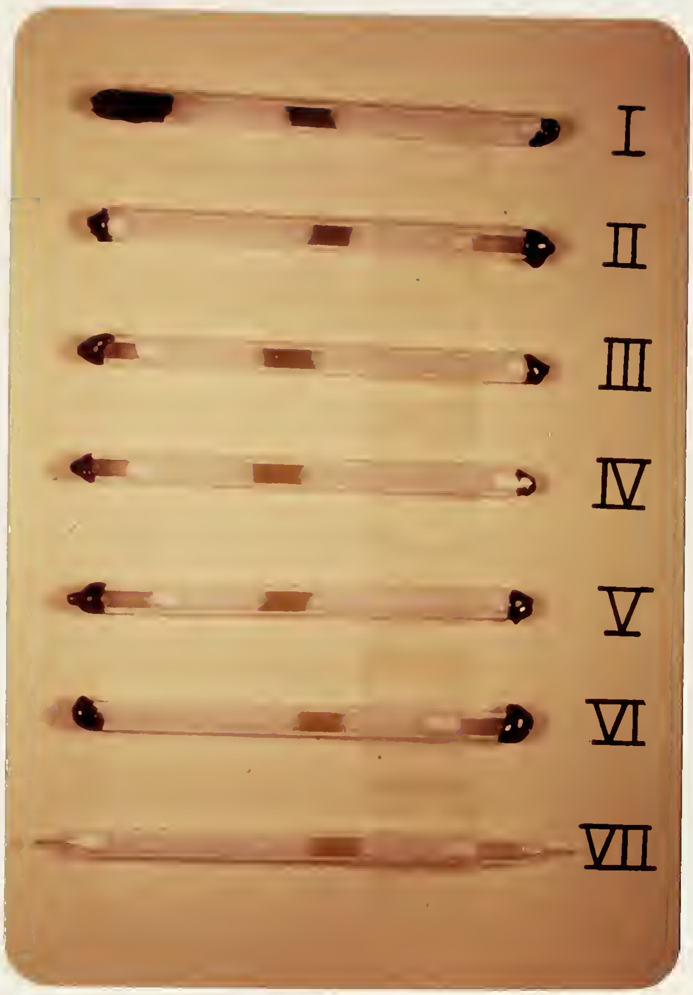
1. Gas sampling flask

2. Carbon monoxide indicating tube in sampling device

3. Rubber-bulb aspirator.

FIGURE 8

COLORIMETRIC INDICATOR TUBES OBTAINED
FROM 1:1 DILUTIONS OF 100% CARBON MONOXIDE
SATURATED CHICK BLOOD



100%

50%

25%

12.5%

6.25%

3.13%

Unexposed

carbon monoxide in blood. This was done as follows:

(a) three guinea pigs, three 4-week old chicks and three adult hens were bled by heart puncture; the blood samples were citrated and pooled by group.

(b) each of the pooled samples of blood was saturated with carbon monoxide by bubbling carbon monoxide gas through the sample for a period of not less than 30 minutes.

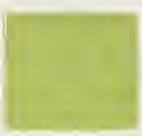
(c) one ml. of each pooled carbon monoxide saturated blood sample was analyzed by the colorimetric method using one aspiration. All three of the pooled blood samples produced an indicator tube color change corresponding to .04 reading on the colorimetric chart (Figure 9). This was taken to represent 100% saturation with carbon monoxide.

(d) one ml. samples of carbon monoxide saturated blood were diluted 1:1 with normal saline solution and tested. In all cases, readings of .02 on the colorimetric chart were obtained (50% HbCO).

(e) one ml. samples of blood from step (d) were diluted 1:1 with normal saline solution, tested, and these gave values of 0.1 on the colorimetric chart (25% HbCO).

FIGURE 9

COLOR STANDARDS AND EQUIVALENT PER CENT HbCO

Color Standard	1 Aspiration	2 Aspirations	3 Aspirations	4 Aspirations
	.1	.05	.033 83%*	.025 63%
	.04 100%	.02 50%	.0133 33.3%	.01 25%
	.02 50%	.01 25%	.0066 16.6%	.005 12.5%
	.01 25%	.005 12.5%	.0033 8.3%	.0025 6.25%
	.005 12.5%	.0025 6.25%	.0016 4.1%	.00125 3.12%
	0	0	0	0

* Percentage figures represent per cent saturation of HbCO

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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
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[illegible]	[illegible]	[illegible]	[illegible]

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(f) one ml. samples of blood from step (e) were diluted 1:1 as above and gave colorimetric readings of .005 (12.5% HbCO).

(g) the second column of figures on the chart was obtained similarly by using two aspirations of the rubber-bulb on gas obtained from 1:1 dilutions of samples (c) to (f) inclusive. The balance of the chart was deduced mathematically.

Once the relationship between indicator color and HbCO concentration had been charted, it was possible to estimate the HbCO in bloods of unknown carbon monoxide content by comparing the color developed by one or more aspirations on the rubber-bulb with the colors developed by known concentrations of HbCO.

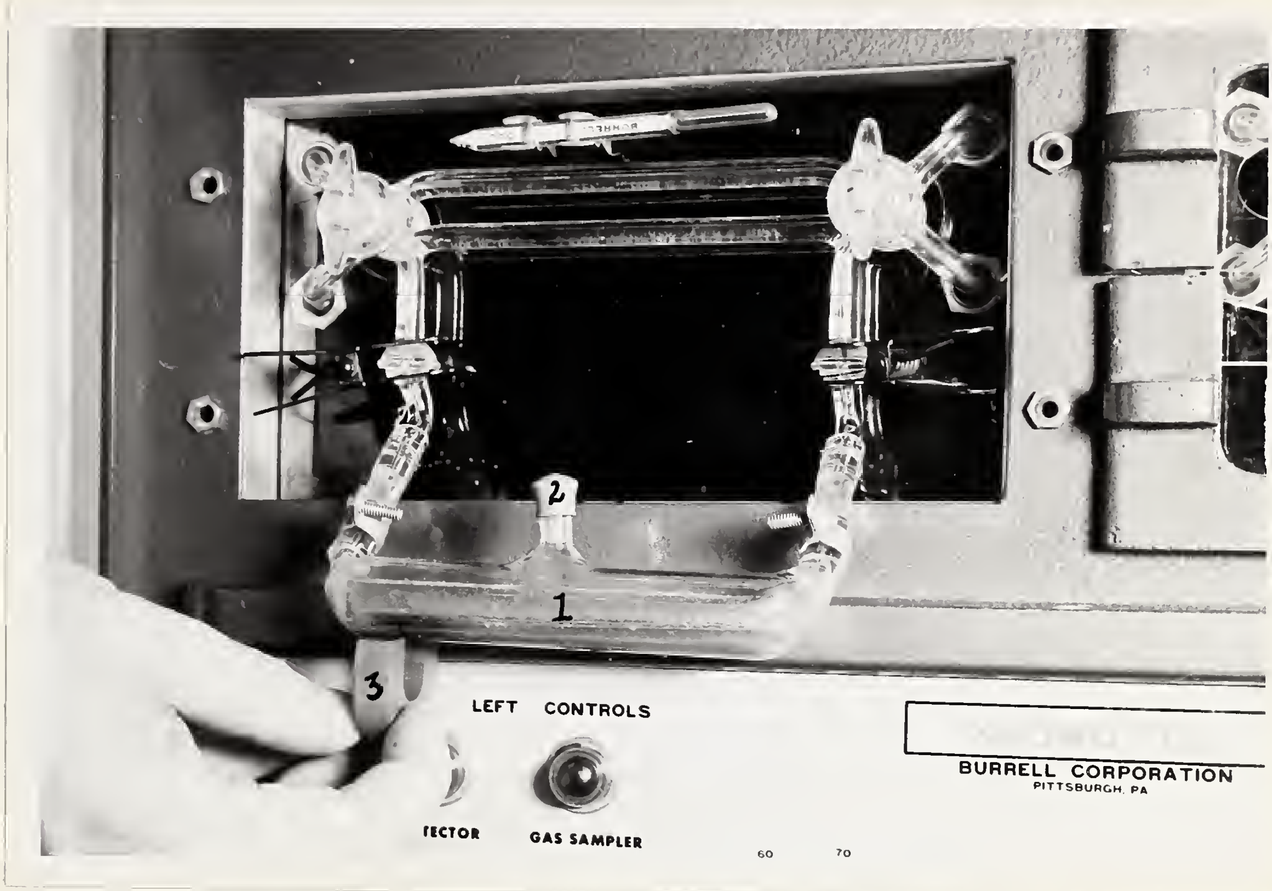
B. Gas Chromatographic Method

A Burrell Kromo-Tog II gas chromatograph was used. A reaction chamber with connections for direct attachment to the gas chromatograph was designed (Figure 10).

To obtain optimum separation of the carbon monoxide from contaminating nitrogen and oxygen, 51 samples containing different proportions of these three gases were injected into the instrument, separated and analyzed. The optimum conditions for separation were

FIGURE 10

ILLUSTRATION OF SPECIAL REACTION CHAMBER
ATTACHED IN THE FLOW STREAM OF THE
KROMO-TOG II GAS CHROMATOGRAPH



1. Reaction chamber

2. Serum cap

3. Magnet

as described in Figures 11, 12, 13 and 14.

Next, the optimum proportion of blood to ferricyanide reagent was determined. This was found to be 1 ml. of blood to 5 ml. of reagent.

The procedure of analysis was as follows:

1. the sample of blood was shaken to secure maximum distribution of the suspended matter.

2. one ml. of blood was injected into the isolated special reaction chamber through the serum cap by means of a 2-ml. hypodermic syringe equipped with a 22-gauge needle.

3. five ml. of the ferricyanide solution was injected into the reaction chamber with a 5-ml. syringe and a 22-gauge needle.

4. a magnet was applied to the outside of the reaction chamber and moved back and forth to cause the four small, steel ball bearings inside the chamber to move and thus mix the blood and ferricyanide reagent. This mixing was continued for a period of 5 minutes.

5. the stopcocks on the gas chromatograph were manipulated and the accumulated gases in the reaction chamber were swept by the helium carrier flow into the chromatographic column.

6. time was allowed for the gas to traverse the column and be recorded graphically on the mechanical

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recording device.

7. the reaction chamber was removed from the chromatograph, washed with water and dried by air under pressure.

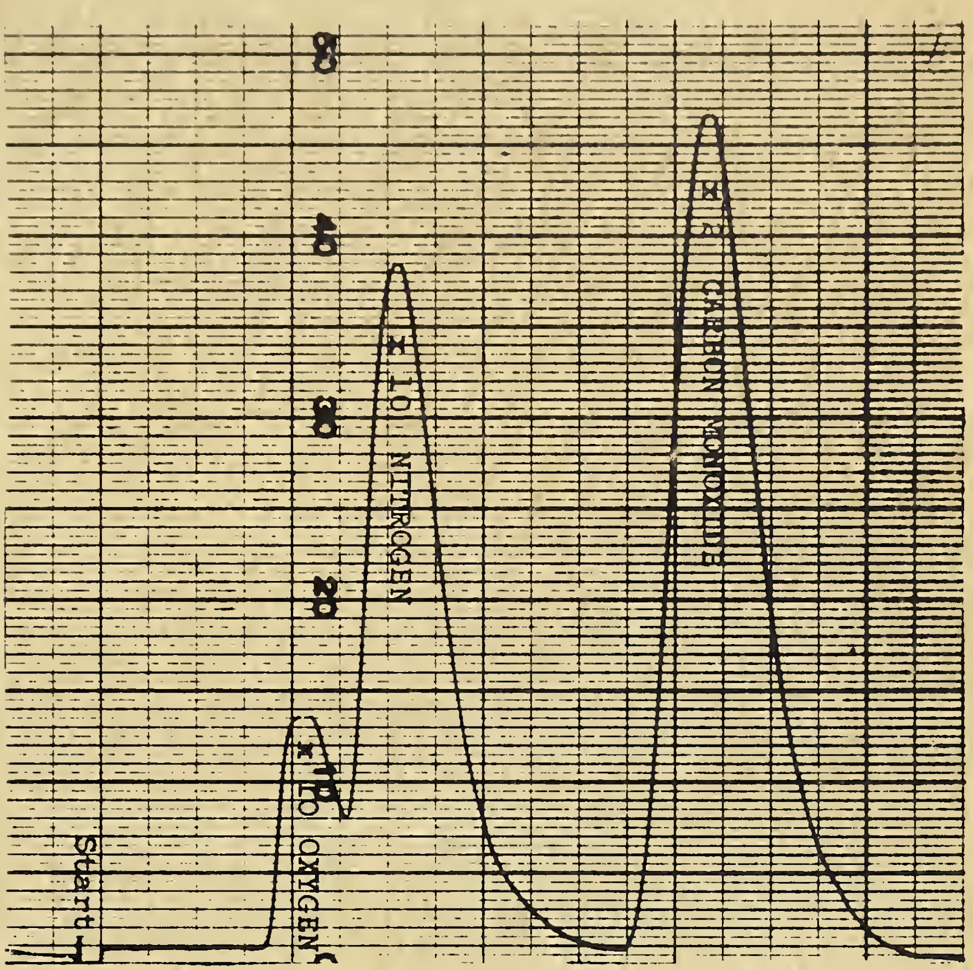
8. the reaction chamber was again attached to the chromatograph and allowed to fill with helium gas. The instrument was then ready to accept another sample of blood for analysis.

In order to permit comparisons between bloods of unknown carbon monoxide content, it was necessary to determine the peak area developed from carbon monoxide saturated blood (assumed to contain 100% HbCO). This was accomplished in the following manner. Three guinea pigs were bled by heart puncture. The blood samples were citrated and pooled. Blood from three 4-week old chicks was similarly prepared. Both samples were saturated to 100% HbCO by bubbling carbon monoxide gas through for a minimum of 30 minutes. Each sample was divided into seven 1 ml. aliquots which were analyzed by gas chromatography for carbon monoxide content. The peak areas attained from the seven analyses of each sample were determined by the use of a planimeter. The average peak area resulting from the analyses of one ml. aliquots of blood from the guinea pigs was 5.66 sq. in. This is closely approximated by the peak shown in Figure 11. It was necessary to record this peak at an

attenuation of two on the recording device, in order that it could be completely drawn on the graph paper. The average peak area resulting from the analyses of the seven samples of chick blood was 2.91 sq. in. An illustration of a typical peak obtained with carbon monoxide saturated chick blood is shown in Figure 12. The average peak areas of 5.66 and 2.91 sq. in. were taken to represent 100% HbCO concentrations for each species. Figure 13 shows the peak area attained from a chick from Experiment 7. The area of this peak was 1.56 sq. in., and therefore represented 53.6% HbCO. Figure 14 shows the peak obtained from the analysis of a guinea pig from Experiment 10. This peak represents a HbCO saturation of 53.4%.

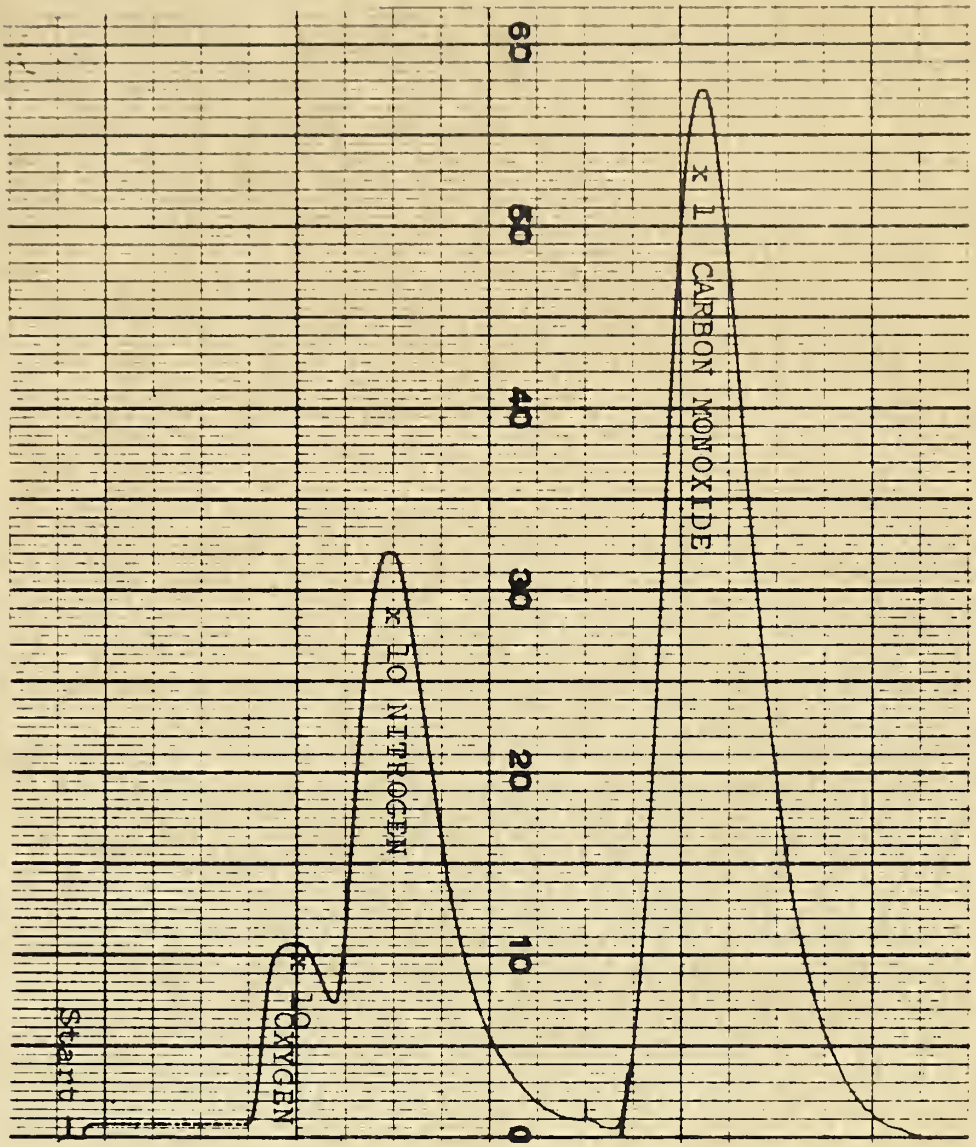
FIGURE 11

OXYGEN, NITROGEN AND CARBON MONOXIDE PEAKS OBTAINED FROM 100% HbCO SATURATED GUINEA PIG BLOOD BY GAS CHROMATOGRAPHIC METHOD



Sample size - 1 ml. blood
Column length - 2½ meters
Column diameter - 5 mm.
Column packing - Linde 5A molecular sieve 30-50 mesh
Column voltage - 45 volts
Column temperature - 95° C.
Carrier gas - helium
Gas flow reference - 10 bubbles per minute
Gas flow measuring - 55 ml./minute
Detector current - 245 milliamperes
Detector bath temperature - 150° C.
Peak area - 2.80 sq. in.

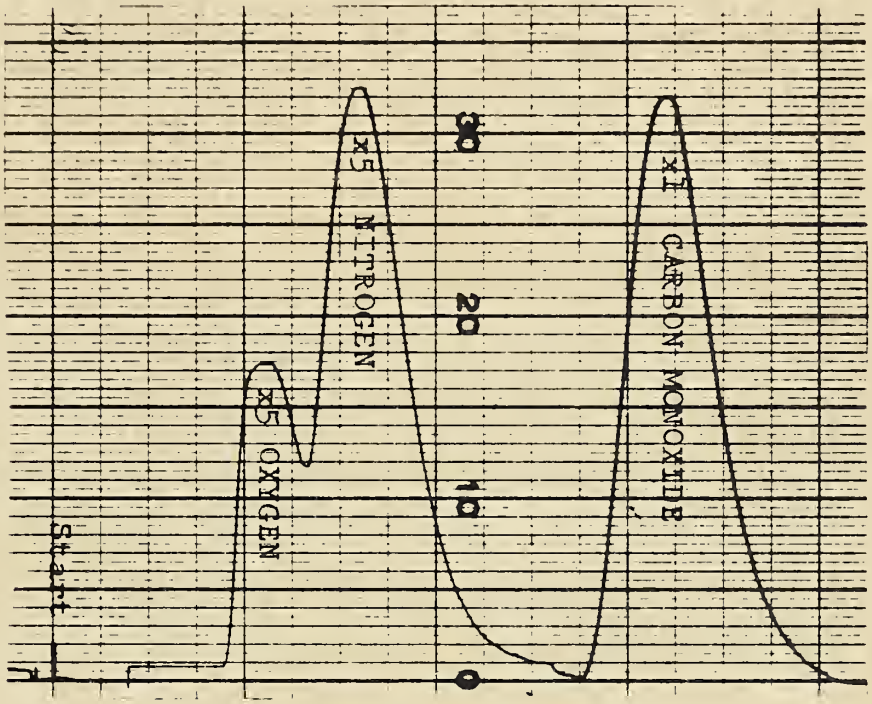
FIGURE 12
OXYGEN, NITROGEN AND CARBON MONOXIDE PEAKS OBTAINED
FROM 100% HbCO SATURATED CHICK BLOOD
BY GAS CHROMATOGRAPHIC METHOD



Sample size - 1 ml. blood
Column length - $2\frac{1}{2}$ meters
Column diameter - 5 mm.
Column packing - Linde 5A molecular sieve 30-50 mesh
Column voltage - 45 volts
Column temperature - 95° C.
Carrier gas - helium
Gas flow reference - 10 bubbles per minute
Gas flow measuring - 55 ml./minute
Detector current - 245 milliamperes
Detector bath temperature - 150° C.
Peak area - 3.01 sq. in.

FIGURE 13

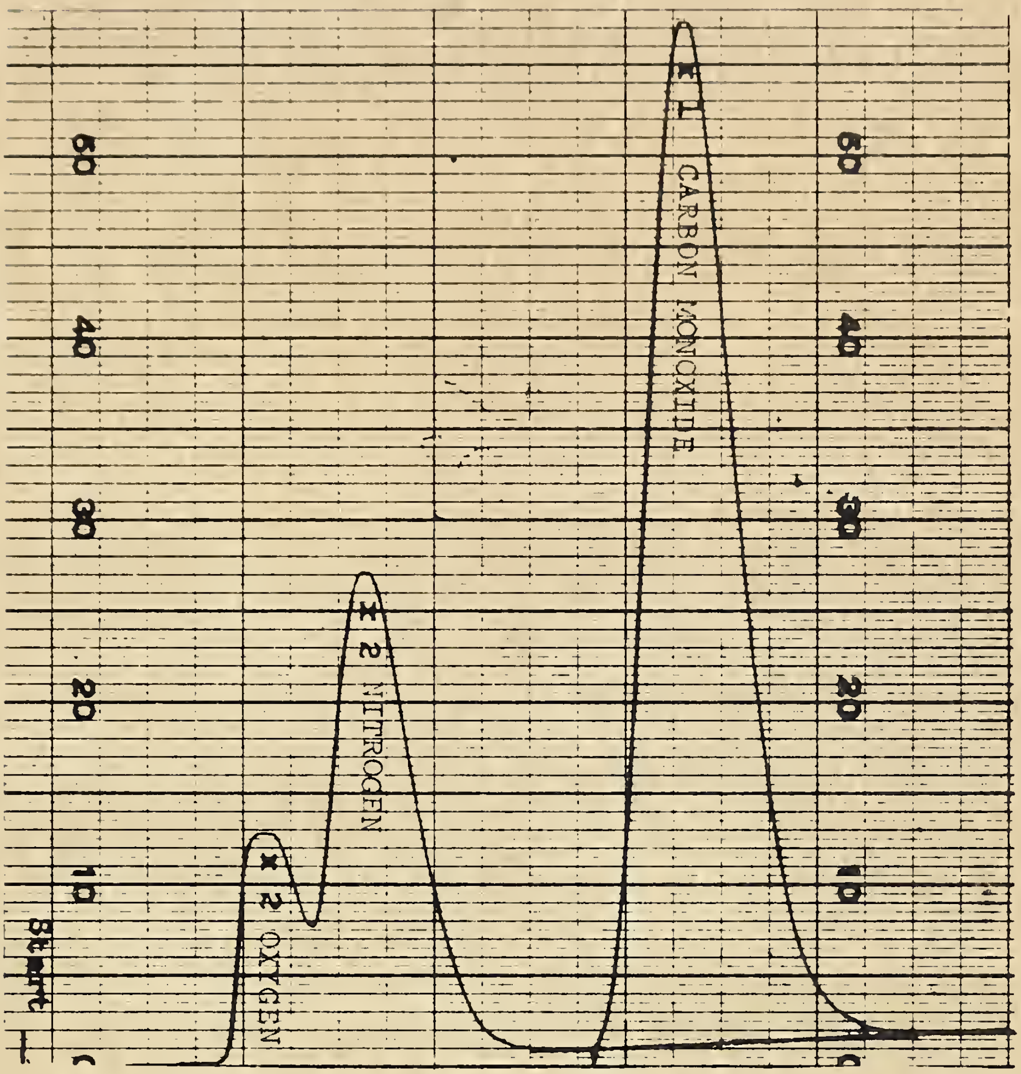
GAS CHROMATOGRAPHIC OXYGEN, NITROGEN AND CARBON MONOXIDE PEAKS OBTAINED ON ANALYSIS OF CHICK BLOOD FROM EXPERIMENT 7



Sample size - 1 ml. blood
Column length - $2\frac{1}{2}$ meters
Column diameter - 5 mm.
Column packing - Linde 5A molecular sieve 30-50 mesh
Column voltage - 45 volts
Column temperature - 95° C.
Carrier gas - helium
Gas flow reference - 10 bubbles per minute
Gas flow measuring - 55 ml./minute
Detector current - 245 milliamperes
Detector bath temperature - 150° C.
Peak area - 1.56 sq. in.

FIGURE 14

GAS CHROMATOGRAPHIC OXYGEN, NITROGEN AND CARBON MONOXIDE PEAKS OBTAINED ON ANALYSIS OF GUINEA PIG BLOOD FROM EXPERIMENT 10



Sample size - 1 ml. blood
Column length - 2½ meters
Column diameter - 5 mm.
Column packing - Linde 5A molecular sieve 30-50 mesh
Column voltage - 45 volts
Column temperature - 95° C.
Carrier gas - helium
Gas flow reference - 10 bubbles per minute
Gas flow measuring - 55 ml./minute
Detector current - 245 milliamperes
Detector bath temperature - 150° C.
Peak area - 3.02 sq. in.